REMARKS

The following remarks are in response to the January 29, 2002 final Office Action ("Office Action") in said parent application.

Upon entry of this amendment, claims 1, 3-7, 9-20, 27-30, and 32 and 33 will be pending. Claim 31 has been canceled as duplicative. Claims 1, 3-7, 9, 11, 13, 16-20, 27-28, 30, and 32 have been amended to correct antecedent bases, grammar, and typographical errors. Support for further amendments to claim 1 is provided at least in page 7, lines 19-25 and the paragraph bridging pages 16-17 to expressly clarify the step of identifying the novel nucleic acid sequence. Support for further amendments to claim 3 is provided at least on page 7, lines 26-28, and page 8, lines 1-4. Support for further amendments to claim 15 is provided at least on page 16, line 5, through page 17, line 18. Support for the amendment to claim 17 is provided in claim 9 as filed, which clarifies the correct product being manipulated. Support for new claim 33 and amendments added to claims 27 and 30 are found in the priority application which is incorporated in its entirety in the pending application as filed, and throughout the specification, including, but not limited to, *e.g.*, on page 16, line 5, through page 17, line 18; on page 18, lines 1-8; on page 8, lines 10-20; on page 18, lines 17-25; on page 8, line 22, through page 9, line 26; on page 15, line 3-16; and throughout page 15, line 18, through page 20, line 5, Table 1, and the Examples. No new matter has been added as a result of these amendments.

In the instant Office Action, the Examiner (i) withdraws claims 28-30 and 32 from consideration, (ii) objects to claims 1, 3-17, and 31, and (iii) rejects various claims as indefinite, as being anticipated by, and as being obvious in view of the prior art. Applicants respond to each of these actions in turn.

Election/Restriction Overcome:

The Examiner has indicated that new claims 28-30 and 32 have been withdrawn as directed to a non-elected invention. Claims 29 and 30 depend from claim 28. Specifically, the Examiner contends that the inventions of groups I and II are unrelated. *See* Office Action, page 3. Under Title 37 C.F.R. § 1.142(a), a restriction is appropriate "if two or more independent and distinct inventions are claimed in a single application." Applicants respectfully submit that the inventions of claims 1, 3-7, 9-20, 27-30, 32 are neither independent nor distinct from each other. Therefore, Applicants traverse the restriction for the following reasons.

First, Applicants direct the Examiner's attention to the original restriction requirement (Paper No. 6) in this application, mailed July 17, 2000. The elected group containing claims 1-20 were stated to be drawn to "a method of screening, *resulting in the identification* of a nucleic acid sequence and the comparison of that sequence to a reference sequence." *See* Paper No. 6, page 2, ¶ 2 (emphasis added). All pending claims are ultimately directed to a method for identifying a novel nucleic acid sequence. Hence, these claims are connected in design, operation and effect. See MPEP 802.01.

Claims 28, 32 and 33 expand different aspects of the invention embodied in claim 1. In the instant application, claims 1, 28, 32 and 33 are directed to methods of screening a population of nucleic acids to identify a novel sequence. These methods include dividing a cDNA population into subpopulations, sequencing or identifying a first nucleic acid sequence in the subpopulation, and comparing that first sequence to a reference nucleic acid sequence to identify a novel nucleic acid sequence. Whereas claim 1 states that the subpopulations are partitioned, claim 32 states that the subpopulations are normalized. As provided in the specification at page 6, lines 4-5 "The normalization takes place by one or more methods of partitioning the nucleic acid population". The main difference between the claims is in which technique is chosen to divide the subpopulations. All other steps mirror each other, often verbatim. Hence, claims 1 and 32, are connected in design, operation and effect, are <u>not</u> independent, and thus, are not candidates for restriction.

Claim 28 expands the identifying step common to claims 1 and 32. Claim 28 proceeds by assembling the sequenced nucleic acids of the input populations. The resulting sequences can then be checked against a reference to determine if it is known or novel. Claims 29 and 30 merely provide a list of preferred partitioning methods.

Finally, the Examiner has given no indication that she is under an undue burden in searching the subject matter of the newly restricted claims. In fact, as noted by the Examiner on page 3 of the instant Office Action, the claims of group I <u>and</u> the claims of group II are classified in class 435, subclass 6. Any search performed by the Examiner would include the subject matter of the claims in both groups I and II.

For the above reasons, Applicants submit that restriction is improper. Reconsideration and withdrawal of this requirement is respectfully requested

Objections Overcome:

The Examiner has objected to claims 1 and 31 as being identical. Applicants have canceled claim 31 in response. The objection is now moot and should be withdrawn.

The Examiner has objected to claims 3-17 as depending from canceled claim 2. Applicants have amended these claims in response. These objections are now moot and should be withdrawn.

The Examiner has objected to claim 6 for containing a typographical error. Applicants have amended claim 6 in response. This objection is now moot and should be withdrawn.

Rejection under 35 U.S.C. § 112, ¶ 2 Overcome:

The Examiner has rejected claims 7 and 27 as indefinite. Specifically, the Examiner states that the claim is confusing as to whether or not the partitioning element is in addition to the partitioning step of claim 1, or if it replaces that step. Applicants submit that either interpretation is within the scope of the invention. For example, the Specification, at page 6, lines 25-27, expressly states: "Unless stated otherwise, any partitioning method described herein can be used in conjunction with one or more additional partitioning methods." Applicants have amended claims 7 and 27 to indicate that the recited partitioning steps may be used either in combination with a restriction digest, or as an option to a restriction digest.

Withdrawal of the rejection is respectfully requested.

Rejection under 35 U.S.C. § 102(e) Overcome:

The Examiner has rejected claims 1, 31, 19, and 27 as being anticipated by Austin *et al.*, U.S. Patent No: 6,132,965 (2000), (hereinafter "Austin"). According to the Examiner, Austin teaches all the elements of claims 1, 31, 19, and 27. The rejection is traversed as applied to the claims as amended.

To anticipate a claim, the reference must teach each and every element of the claimed invention. Claim 1 as amended, from which claims 19 and 27 depend, is drawn to a method of screening a population of nucleic acids for a novel sequence, partitioning the population by digestion with one or more restriction enzymes into subpopulations, sequencing at least one

nucleic acid sequence in the subpopulation to provide a first nucleic acid sequence, and determining whether the first nucleic acid sequence is novel..

In contrast, the language that the Examiner cites from the Austin patent pertains to conventional subtractive hybridization which is used in Austin to identify preferentially expressed cDNA to provide genetic and immunological markers for the diagnosis, therapy and treatment of homocysteine-induced vascular disease. As discussed by Austin, column 22, lines 40-66, subtractive hybridization is used to enrich for particular, known nucleic acids , and entails hybridizing two pools (driver and tester) of cDNA to each other so as to identify and separate those cDNAs that do not hybridize and, thus, are not represented in one of the samples.

In contrast, Applicants' invention generates collections of long or short fragments depending on the specifically partitioned cDNA molecules to provide a diversity of nucleic acid sequences. Claim 1 simply does not teach conventional subtractive hybridization, enrichment for the known sequence or enhancement of hybridization as taught in Austin. As provided in the specification:

> ...transcripts from as few as 10-15 gene may represent 10-15% of cellular mRNA by mass. In addition to these highly abundant transcripts, another 1000-2000 genes encode moderately abundant transcripts, which can account for up to 50% of cellular mRNA mass. Transripts from the remaining gene fall into the low abundance class.
>
> Because many genes are identified by isolating complementary DNA (cDNA) corresponding to an RNA sequence, a significant problem can arise because of differences in the levels at which specific RNAs are present in cell types. The most abundant sequences can be repeatedly sampled, while the lowest abundance class may be rarely, if ever, sampled.

(See specification at page 1, lines 11-18)

Austin fails to teach one of ordinary skill in the art to partition a cDNA population into one or more subpopulations, or to sequence a first nucleic acid sequence in the subpopulation to establish the novelty of the sequence. Furthermore, Austin fails to teach the skilled artisan to solve the problem of identifying **novel** sequences.

As the Examiner is clearly aware, the dictionary definition of "novel" as used in the art and as utilized in the claims (e.g., in claim 1 "...A method of screening a population of nucleic acids for a **novel** sequence..." and "...whereby the first nucleic acid sequence is **novel** if it is not identical to a reference nucleic acid sequence.") refers to those sequences which are "new and

not resembling something formerly known or used" (See, Appendix B – Merriam-Webster OnLine, definition for "novel" - Main Entry). The novel sequences of the claimed invention are not predetermined or known in advance or isolated as required in the methods of Austin.

Because Austin fails to disclose **all the limitations** of claim 1, it does not anticipate claim 1 or the claims dependent thereon. Likewise, claims 19 and 27, which depend directly from claim 1, are not anticipated. Claim 31 has been cancelled and the rejection is now moot as to claim 31.

Applicants respectfully request that the rejection be withdrawn.

Rejection under 35 U.S.C. § 103(a) Overcome:

Claims 3-7, 18 and 20 are rejected by the Examiner as being obvious in view of Austin. The Examiner concedes that Austin does not teach multiple aspects of the claimed invention, including a) partitioning the population of RNA molecules before creating cDNA molecules; b) from what specific portion the cDNA is derived, *i.e.*, 5' end, interior, or 3' end, of the RNA molecule ; c) hybridization of a probe nucleic acid sequence to the population of nucleic acids; d) comparing sequences by determining at least a portion of the nucleotide sequence of the first nucleic acid sequence and comparing that to a reference nucleic acid sequence; or e) equalizing the representation of the molecules. *See* Office Action, the paragraph bridging pages 7-8.

Austin fails to suggest or motivate one of ordinary skill in the art to partition a cDNA population into one or more subpopulations, or to sequence a first nucleic acid sequence in the subpopulation to establish the novelty of the sequence much less provide the deficiencies the Examiner has alleged. Nowhere is there a suggestion within Austin to modify the reference to achieve the invention as claimed.

In order to fill the gap, the Examiner relies on the statement that the above elements would have been obvious to one of ordinary skill in the art at the time the invention was filed. *See* Office Action, pages 8-9. Yet, the Examiner provides no evidence to support her conclusory statement much less provide objective evidence that the elements missing from Austin are not only known in the prior art, but are known in the combination asclaimed.

Mere statements or reliance on common knowledge in the art do not suffice to support a rejection or establish a prima facie case of obviousness. Applicants traverse this rejection as

applied to the claims as amended, and request that the Examiner cite references, provide an affidavit or objective evidence in support of her position, pursuant to MPEP § 2144.03. (See also, In re Fine 5 USPQ2d 1596 (CAFC 1988) and In re Dembiczak, 50 USPQ2d 1614 (CAFC 1998). Prior cases and the Administative Procedures Act have established that the PTO must provide authority of objective evidence articulated and placed on the record. Conclusory statements of what is "basic knowledge" do not fulfill the agency's obligations and is not consistent with effective administrative procedure or review. (See, in re Lee, 61 USPQ2D 1430, 1435 (CAFC 2002) Since Austin alone does not provide one of ordinary skill in the art the motivation or suggestion to make Applicants' claimed invention, the Examiners conclusory assertions of "desirability" or "advantageous" methods falls far short of supporting an obviousness rejection.

Since claim 1 of the instant application solves a different problem than that addressed in Austin, claim 1 and the claims dependent thereon are not obvious. Austin does not teach and does not suggest many of the aspects of the instant invention. Thus, for the reasons discussed above, claim 3 (directed to partitioning RNA molecules), claim 4 (directed to partitioning from the 5' end), claim 5 (directed to cDNA derived from interior regions), claim 6 (directed to cDNA derived from the 3' ends) and claim 7 (directed to partitioning by hybridization) are patentably nonobvious over Austin.

The Examiner concedes that Austin does not teach comparing sequences by determining a portion of the nucleotide sequences of a first nucleic acid sequence and a reference nucleic acid sequence, *see* Office Action, page 8-9. However, the Examiner does not develop the reasoning as to why claim 18 is allegedly obvious in view of Austin. Thus, the Examiner has no foundation upon which to reject claim 18. Applicants request that the Examiner supply the rationale upon which she relies, so that a clear issue can be developed.

For the reasons discussed above, Austin fails to supply or suggest the missing elements and steps in independent claim 20 much less supply them in the order as required in claim 20. Nor does the Examiner's conclusory statements fill that gap.

The rejection of claims 3-7, 18 and 20 is improper and must be withdrawn.

Claims 9-17 are nonobvious under 35 U.S.C. § 103(a) over Austin and further in view of Sytkowski:

The Examiner additionally rejects claims 9-17 as unpatentable over Austin and further in view of Sytkowski *et al.*, U.S. Patent No. 6,177,244 (2001) (hereinafter "Sytkowski"). Claims 9-17 are directly or indirectly dependent from claim 1. Applicants traverse this rejection as applied to claims 9-17 as amended.

As noted above, the Examiner acknowledges that multiple elements are missing from Austin and concedes that Austin does not teach many addition elements, including: ligating adapter oligonucleotides to the termini of digested cDNA molecules (claim 9), amplifying those products (claim 10), separating them using gel electrophoresis (claims 11, 12 and 15), comparing the sizes of the populations (claims 13 and 16), recovering the size-separated products and reamplifying them (claim 14), or inserting the ligated adapter oligonucleotide into a cloning vector to form a vector-insert; transforming the vector-insert into a suitable host, recovering the vector-insert from said host, and digesting the vector-insert with one or more restriction enzymes, thereby releasing said insert; and comparing the size of the insert to sizes of fragments generated by the same restriction enzyme or enzymes in said nucleic acid (claim 17). The Examiner's use of Sytkowski does not overcome these deficiencies.

The technique disclosed in Sytkowski is based on subtractive hybridization, the same technique disclosed in Austin. This combination simply does not produce the invention embodied in claims 9-17. In addition, the Austin and Sytkowski references take mutually exclusive paths and reach different solutions to a similar problem. Since they teach away from each other, it would be totally illogical for one of ordinary skill in the art to combine them. For the reasons discussed above, neither Austin and Sytkowski, alone or in combination, support the rejection under §103.

For the above reasons, Applicants submit that claims 9-17 are patentably nonobvious over Austin and further in view of Sytkowski. Reconsideration and withdrawal of the rejection is respectfully requested.

CONCLUSION

Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,

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Dated: July 29, 2002

Appendix A: Marked Up version Showing Changes Made

Amend the claims as indicated below:

1. (Twice Amended) A method of screening a population of nucleic acids [for] to identify a novel sequence, the method comprising:

providing a population of cDNA molecules derived from a population of RNA molecules;

partitioning said cDNA population into one or more subpopulations of nucleic acid[s] sequences,

wherein said partitioning step comprises digesting the cDNA molecules with one or more restriction enzymes;

[identifying a first nucleic acid sequence in the subpopulation of nucleic acid sequences] sequencing at least one nucleic acid sequence in the subpopulation to provide a first nucleic acid sequence; and

[comparing] determining whether the first nucleic acid sequence is identical to a reference nucleic acid sequence [or sequences, wherein the absence of the first nucleic acid sequence in the reference nucleic acid or nucleic acid sequences indicates] ,whereby the first nucleic acid is [a] novel [nucleic acid sequence] if the first nucleic acid sequence is not identical to the reference nucleic acid sequence.

3. (Amended) The method of claim [2] 1, further comprising the step of partitioning the population of RNA molecules before providing the population of cDNA molecules.

4. (Amended) The method of claim [2] 1, wherein said cDNA population is derived from the 5' ends of the RNA molecules.

5. (Amended) The method of claim [2] 1, wherein said cDNA population is derived from the interior regions of the RNA molecules.

6. (Amended) The method of claim [2] 1, wherein said cDNA population is derived from the 3' ends of the [DNA] RNA molecules.

7. (Amended) The method of claim [2] 1, wherein said partitioning step optionally comprises [hybridization] hybridizing [of] a probe nucleic acid sequence to the population of nucleic acids.

9. (Amended) The method of claim [8] 1, further comprising ligating adapter oligonucleotides to the termini of the digested cDNA molecules, thereby producing ligation products.

11. (Amended) The method of claim [8] 10, further comprising separating the amplified products.

13. (Amended) The method of claim 11, wherein the first nucleic acid sequence is identified by comparing the size of one or more digestion products produced by a member of the subpopulation of nucleic acid[s] sequences to the sizes of fragments generated by the same restriction enzyme or enzymes in said reference nucleic acid or nucleic acids.

15. (Amended) The method of claim 14, wherein said separating is by gel electrophoresis or liquid chromatography.

16. (Amended) The method of claim 15, wherein the first nucleic acid sequence is identified by comparing the size of one or more digestion products produced by a member of the subpopulation of nucleic acid[s] sequences to the sizes of fragments generated by the same restriction enzyme [or enzymes] in said reference nucleic acid [or nucleic acids] sequences.

17. (Amended) The method of claim 9, further comprising:

inserting the [ligated adapter oligonucleotide] ligation product into a cloning vector to form a vector-insert;

transforming the vector-insert into a suitable host;

culturing [transformed] the host under conditions allowing for replication of the vector-insert;

recovering the vector-insert from said host; and

digesting the vector-insert with one or more restriction enzymes, thereby releasing said insert; and

comparing the size of the insert to sizes of fragments generated by the same restriction enzyme or enzymes in said reference nucleic acid or nucleic acids.

18. (Amended) The method of claim 1, wherein [comparing is by determining] at least a portion of the [nucleotide sequence of the] first nucleic acid sequence is determined and [comparing the] compared to nucleotide sequence to the nucleotide sequence of one or more reference nucleic acid[s] sequences.

19. (Amended) The method of claim 1, wherein [comparing is by] the determining step comprises hybridizing the first nucleic acid sequence to one or more of the reference nucleic acid sequences.

20. (Twice Amended) A method for equalizing the representation of nucleic acid[s] sequence in a population of nucleic acid[s] sequences, the method comprising in order the steps of:

providing a population of cDNA molecules derived from a population of RNA molecules, wherein said cDNA population comprises a first nucleic acid and a second nucleic acid sequence having a nucleic acid sequence distinct from the first nucleic acid sequence, and wherein said first nucleic acid sequence is present at a higher level in said population than said second [population] nucleic acid sequences;

partitioning said cDNA population into one or more subpopulations of nucleic acid[s] sequences,

wherein said partitioning comprises digesting the cDNA [molecules] population with one or more restriction enzymes; and

lowering the level of said first nucleic acid sequence relative to the level of said second nucleic acid sequence in the subpopulation of nucleic acid sequences, thereby equalizing the representation of nucleic acid[s] sequences in said population of nucleic acid[s] sequences.

27. (Amended) The method of claim 1, wherein the partitioning step optionally comprises one or more processes [chosen from the group consisting of] selected from:

a) isolating nucleic acid[s] sequences from different cell types;

b) separating the nucleic acid[s] sequences in the subpopulation by [size] physical properties;

c) amplification [that provides] of a specific [a] subpopulation of nucleic acid[s] sequences;

d) [preferentially] amplifying 5' terminal sequences of the nucleic acid[s] sequences;

e) [preferentially] amplifying interior sequences of the nucleic acid[s] sequences; and

f) [preferentially] amplifying 3' terminal sequences of the nucleic acid[s] sequences;

g) partitioned subtraction screening,

h) mass spectroscopy,

i) length selection by lariat formation,

j) use of identical primers,

k) use of shortened primers,

l) use of intermediate annealing temperature,

m) use of modified cycle times, and

n) incremental batch assembly.

28. (Amended) A method of identifying a novel nucleic acid sequence, the method comprising:

providing a population of nucleic acid molecules;

normalizing the population to provide one or more subpopulations of nucleic acid[s] sequences;

[determining the sequence of] sequencing a plurality of nucleic acid [molecules] sequences in the one or more subpopulations;

assembling [a] the plurality of nucleic acid sequences to provide an assembled sequence; and

determining whether the assembled sequence is absent in a reference set of one or more reference nucleic acid sequences;

whereby if the [absence of the] assembled sequence is absent from the reference[indicates] the set assembled sequence is a novel nucleic acid sequence.

30. (Amended) The method of claim 29, wherein the partitioning comprises one or more processes [chosen from the group consisting of] selected from:

a) isolating nucleic acid[s] sequences from different cell types,

b) separating the nucleic acid[s] sequences in the subpopulation by size,

c) amplification [that provides a] of at least one subpopulation of nucleic acid[s] sequences,

d) [preferentially] amplifying 5' terminal sequences of the nucleic acid[s] sequences,

e) [preferentially] amplifying interior sequences of the nucleic acid[s] sequences,

f) [preferentially] amplifying 3' terminal sequences of the nucleic acid[s] sequences, and

g) hybridization of said population against a prepared library of known nucleotide sequences;

h) partitioned subtraction screening,

i) mass spectroscopy,

j) length selection by lariat formation,

k) use of identical primers,

l) use of shortened primers,

m) use of intermediate annealing temperature,

n) use of modified cycle times, and

o) incremental batch assembly.

32. (Amended) A method of screening a population of nucleic acid [for] molecules to identify a novel sequence, the method comprising:

providing a population of nucleic acid sequences;

normalizing said population into one or more subpopulations of nucleic acid[s] sequences, wherein said normalizing is selected from the group consisting of restriction endonuclease digestion, size-based fragment [partioning] partitioning; terminal nucleotide sequence, and fragment migratory pattern;

identifying a first nucleic acid sequence in the subpopulation of nucleic acid sequences; and

comparing the first nucleic acid sequence to a reference nucleic acid sequence or sequences, wherein the absence of the first nucleic acid sequence in the reference nucleic acid sequence or nucleic acid sequences indicates the first nucleic acid sequence is a novel nucleic acid sequence.

Add new claim 33.

--33. The method of screening as in claim 33 wherein the normalization step comprises processes selected from the group consisting of partitioned subtraction screening, mass spectroscopy, length selection by lariat formation, use of identical primers, use of shortened primers, use of intermediate annealing temperature, use of modified cycle times, use of a 5'-capped end and incremental batch assembly. --

Appendix B: Merriam Webster OnLine

http://www.m-w.com/cgi-bin/dictionary

5 entries found for **novel**.

To select an entry, click on it.

novel[1,adjective]
novel[2,noun]
dime novel
graphic novel
saga novel

Go

Main Entry: **[1]nov·el**

Pronunciation: 'nä-v&l

Function: *adjective*

Etymology: Middle English, from Middle French, new, from Latin *novellus,* from diminutive of *novus* new -- more at NEW

Date: 15th century

1 : new and not resembling something formerly known or used

2 : original or striking especially in conception or style <a *novel* scheme to collect money>

synonym see NEW